[Chem. Pharm. Bull. 26(7)2198—2204(1978)]

UDC 547.466.1.04 : 547.269.3.04

Selective Cleavage of *tert*-Butyloxycarbonyl Protecting Group with Dilute Organic Sulfonic Acid without Decomposition of Tryptophan Residue[1,2]

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(Received February 22, 1978)

The reagent of 2 M anhydrous *p*-toluenesulfonic acid in dioxane containing 2% anisole cleaves selectively *tert*-butyloxycarbonyl group in Boc-Trp-OH without any detectable decomposition of tryptophan residue. Another reagents such as dilute *p*-toluenesulfonic acid, methanesulfonic acid or ethanesulfonic acid in acetic acid and methanesulfonic acid or ethanesulfonic acid in dioxane, which are containing 2% anisole and 2% 1,2-ethanedithiol or triphenylphosphine as reducing reagent, have also similar properties to those of *p*-toluenesulfonic acid in dioxane containing 2% anisole. The utility of the reagents was proved as applied to the syntheses of three kinds of tryptophan containing peptide derivatives by the classical solution method.

Keywords——tryptophan; *p*-toluenesulfonic acid; methanesulfonic acid; ethanesulfonic acid; 1,2-ethanedithiol; triphenylphosphine; tryptophan-containing peptide synthesis; Merrifield's resin

The partial decomposition of tryptophan residue is one of the most serious problems during the acidolytic selective cleavage of the Boc group, which is widely used for the protection of amino group. Recently, 2-mercaptoethanesulfonic acid in acetic acid have been advocated for the selective removal of the Boc group from tryptophan containing peptide derivatives.[4] The cleavage of the Boc group in Boc-peptide derivatives with the use of dilute organic sulfonic acids has been reported recently.[5] However, the properties to tryptophan residue have not been studied in detail yet.

The study described in this paper concerns with minimization of the decomposition of tryptophan residue with the use of easily available organic sulfonic acid reagents for the selective acidolytic cleavage of the Boc group in Boc-Trp-OH and peptide derivatives containing tryptophan residue. The properties of the reagents to various protecting groups used widely in peptide synthesis and the synthesis of tryptophan containing peptide derivatives with the reagents are also described.

1) A part of this work was presented at the 49th Annual Meeting of the Japanese Biochemical Society, Sapporo, September 1976; K. Suzuki, N. Endo, K. Nitta and Y. Sasaki, *Seikagaku*, **48**, 424 (1976), and at the 26 th IUPAC Congress, Tokyo, September 1977; K. Suzuki, N. Endo, K. Nitta and Y. Sasaki, Abstracts, p. 1239 (1977).

2) Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: Boc=*t*-butyloxycarbonyl, TsOH=anhydrous *p*-toluenesulfonic acid, MsOH=methanesulfonic acid, EsOH= ethanesulfonic acid, EDT=1,2-ethanedithiol, TPP=triphenylphosphine, UV=ultraviolet, IR=infrared, MS=mass spectrum, Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, TFA=trifluoroacetic acid, MBS=*p*-methoxybenzenesulfonyl, ONb=*p*-nitrobenzyl ester, OBzl=benzyl ester, OEt=ethyl ester, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DMF=dimethylformamide, ONp=*p*-nitrophenyl ester. The -resin represents ester bond derived from N-protected amino acid or peptide with chloromethylated polystyrene 2% divinylbenzene.

3) Location: *Komatsushima, Sendai 983, Japan.*

4) A. Loffet and C. Dremier, *Experientia*, **27**, 1003 (1971).

5) *a*) J. Goodacer, R.J. Ponsford and I. Stirling, *Tetrahedron Lett.*, **1975**, 3069; *b*) H. Yajima, H. Ogawa, N. Fujii and S. Funakoshi, *Chem. Pharm. Bull.* (Tokyo), **25**, 740 (1977).

Boc-Trp-OH was treated with 10 molar excess of 2 M TsOH in dioxane containing 2% anisole as a cation scavenger at room temperature for 5 minutes to 24 hours, and a part of the reaction mixture which was neutralized with 1 N sodium bicarbonate was submitted to quantitative amino acid analysis. Tryptophan was recovered quantitatively within 30 minutes, and even after 24 hours within experimental error. The other dilute organic sulfonic acid reagents shown in Table I have quite similar properties with 2 M TsOH in dioxane containing

Table I. Reagent for the Selective Cleavage of Boc Group from Boc-Trp-OH and Recoveries of Trp

Sulfonic acid	Concentration (M)	Solvent	Additive(s)[a]	Recovery (%)			
				5 min	15 min	30 min	24 hr
TsOH[b]	2	Dioxane	2% anisole	96	100	102	104
TsOH	1.5	Acetic acid	2% anisole and 2% EDT		92	99	102
MsOH	2.5	Dioxane	Ditto	95	94	100	102
MsOH	3	Acetic acid	Ditto	96	98	100	102
EsOH	3	Dioxane	Ditto		95	100	98
EsOH	3	Acetic acid	Ditto		93	98	100
TsOH	1.5	Acetic acid	2% anisole and 2% TPP			99	83
MsOH	2.5	Dioxane	Ditto			100	81
MsOH	3	Acetic acid	Ditto			106	79
EsOH	3	Dioxane	Ditto			97	
EsOH	3	Acetic acid	Ditto			103	

a) Additive(s) were added just before use.
b) TsOH in dioxane can be used without any detectable decomposition of tryptophan residue, after the storage in brown bottle for several months at room temperature.

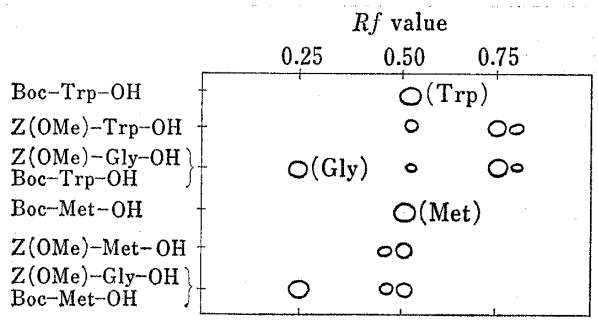
anisole, but addition of EDT[6] or TPP[7] as reducing reagent was required. The recoveries of tryptophan from the reaction mixture of sulfonic acid containing TPP with Boc-Trp-OH were quantitative within 30 minutes and about 80% after 24 hours respectively. The reason of the lower recoveries after 24 hours was proved due to instability of TPP in the sulfonic acid reagents, suggesting that TPP containing reagents should be prepared freshly. All of the reagents shown in Table I were freely soluble in ether.

Further confirmations were made to be recovered intact tryptophan quantitatively from Boc-Trp-OH with the use of two reagents as example. Thus, the reaction mixture of Boc-Trp-OH with 2 M TsOH in dioxane containing 2% anisole or 3 M MsOH in acetic acid containing 2% anisole and 2% EDT at room temperature for 30 minutes was added ether and the precipitate thereby formed was washed with ether. The water solution of the precipitate was passed through a column of Dowex 1×2 (acetate form) to remove the sulfonic acid and the fractions positive to ninhydrin and Ehrlich reagents were pooled, evaporated and dried over potassium hydroxide in vacuum. The yield of tryptophan thus derived from Boc-Trp-OH was almost quantitative. The physical and chemical properties of the product, namely, paper chromatogram, paper electrophoresis, melting point, specific rotation, UV spectrum, IR spectrum and MS, were quite identical with those of authentic tryptophan. Thus, it was further confirmed that the reaction of Boc-Trp-OH with the dilute organic sulfonic acid reagents investigated above gave intact tryptophan quantitatively.

The properties of the reagents to various protecting groups used widely in peptide chemistry are quite similar to those of dilute sulfonic acid in acetic acid reagents reported by

6) J.J. Sharp, A.B. Robinson and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973). When EDT containing sample was determined on a short column of amino acid analyzer, a peak due to purplish red product of EDT with ninhydrin was detected prior to the elution position of tryptophan.
7) M. Ueki and S. Ikeda, *Chem. Lett.*, **1977**, 869.

Yajima, *et al.*[5b] On the contrary to the acetic acid solution, the acidolytic cleavage of the Z group in Boc-Lys(Z)-OH with 2 M TsOH, 2.5 M MsOH or 3 M EsOH in dioxane reagent was not observed within 24 hours' on-standing. Additionally, ester bond of amino acid with Merrifield's resin was also more stable to these reagents in dioxane as compared with 50% TFA in methylenechloride. However, it is noteworthy to note that the treatment of Z(OMe)-Trp-OH with 2 M TsOH in dioxane containing 2% anisole or some of the other dilute sulfonic acid shown in Table I (1.5 M TsOH in acetic acid–2% anisole and 2% EDT, 2.5 M MsOH in dioxane–2% anisole and 2% EDT, 1.5 M TsOH in acetic acid–2% anisole and 2% TPP, 2.5 M MsOH in dioxane–2% anisole and 2% TPP, 3 M MsOH in acetic acid 2% anisole and 2% TPP and 3 M EsOH in dioxane–2% anisole and 2% TPP) give much by-products as analyzed by paper chromatography. An example of chromatograms with the use of 2 M TsOH in dioxane containing 2% anisole were shown in Fig. 1. The by-products were also detected when Boc-Trp-OH was treated together with Z(OMe)-Gly-OH in these reagents. Methionine residue was also much more decomposed in the presence of Z(OMe) group with these dilute sulfonic acid reagents. When thioanisole[8] was used as cation scavenger instead of anisole, rate of formation of the by-products of tryptophan decreased to some extent, but no remarkable decrease of the by-products of methionine was observed. These findings indicate that Z(OMe)-peptide derivative containing tryptophan or methionine residue can not be treated with these dilute sulfonic acid reagents because of the partial decomposition of tryptophan or methionine residue.



Solvent: BuOH–AcOH–pyridine–H_2O (15: 3: 10: 12)

Fig. 1. Paper Chromatographic Pattern of Boc- or Z(OMe)-amino Acids Treated with 2 M TsOH in Dioxane Containing Anisole for 30 min

Swelling characteristics of Boc-Val-resin in sulfonic acid reagents in dioxane shown in Table I were found to be almost the same to those of 50% TFA in methylenechloride which was currently used for the selective cleavage of the Boc group in the solid phase peptide synthesis.[9] Quantitative cleavage of the Boc group in Boc-Gly- or Boc-Val-resin with 2 M TsOH or 2.5 M MsOH in dioxane containing 2% anisole was found to be achieved within 30 minutes, when the liberated amino group was determined by the procedure given by Hancock, *et al.*[10] The results were shown in Table II.

Table II. Cleavage of Boc Group in Boc-amino Acyl-resin by Sulfonic Acid Reagents

Boc-amino acyl-resin	Sulfonic acid	Rate of liberated amino group (%)		
		15 min	30 min	60 min
Boc-Gly-resin (0.122 mmol/g)	2 M TsOH in dioxane	77	100	97
	2.5 M MsOH in dioxane	75	98	96
Boc-Val-resin (0.339 mmol/g)	2 M TsOH in dioxane	56	96	98
	2.5 M MsOH in dioxane	85	98	95

8) W. Bauer and J. Pless, "Peptides: Chemistry, Structure and Biology," ed. by R. Walter and J. Meienhofer, Ann Arbor Sci. Publ. Inc., Ann Arbor, 1975, p. 341.
9) J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and Co., San Francisco, 1969, p. 51.
10) W.S. Hancock, J.E. Battersby and D.R. K.Harding, *Anal. Biochem.*, **69**, 497 (1975).

On the basis of these observations, these reagents were applied for the peptide synthesis. A fully protected pentapeptide, which is a key intermediate for the synthesis of encephalitogenic fragments of myelin protein, has been synthesized by the solution method. The synthetic route for the protected peptide is shown in Chart 1. Boc group in Boc-Trp-

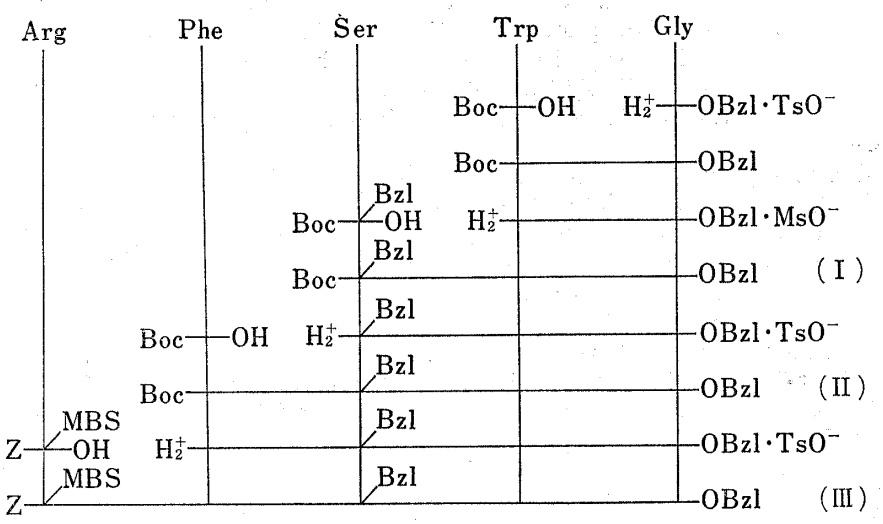


Chart 1. Synthetic Route for the Protected Peptide

Gly-OBzl[11] was removed with 3 M MsOH in acetic acid containing 2% anisole and 2% EDT to assess the utility of the reagent, but the Boc group in all of the other compounds was removed with 5 to 10 molar excess of 2 M TsOH in dioxane containing 2% anisole to avoid the use of stinking EDT. And the resulting tosylates of peptide derivatives were precipitated by the addition of ether. The precipitates formed were often oil which were collected by centrifugation. Peptide bond formation reaction was carried out in the presence of HONB with N,N′-dicyclohexylcarbodiimide (DCC).[12] Similarly, Boc-Phe-Arg(NO_2)-Trp-Gly-OEt, which constitutes positions 7 to 10 in corticotropin, and Boc-Gln-Lys(Z)-Trp-Ala-Pro-OBzl, which is an intermediate for the synthesis of an analog of <Glu-Lys-Trp-Ala-Pro-OH shown inhibitory effect of angiotensin converting enzyme,[13] were prepared with the use of 1-hydroxybenzotriazole (HOBt)[14] instead of HONB in good yields without any remarkable difficulties in the purification process.

We believe that these reagents for the selective cleavage of the Boc protecting group may have a marked validity for the synthesis of tryptophan containing peptide by either the classical solution or the solid phase method. For the classical solution method of peptide synthesis, the organic sulfonic acid reagents shown in Table I give scope for the choice of suitable one due to the variables of the properties of substrate, for example the solubility, and the sulfonates, for example the crystallinity. In a practical standpoint, the use of TPP as one of additives is preferable rather than that of stinking EDT. One difficulty may arise to precipitate a sulfonate of the deblocked peptide quantitatively with ether, if the sulfonate is partially soluble in ether. Tosylate of H-Ser(Bzl)-Trp-Gly-OBzl derived from I is an example such a case. For the solid phase synthesis, the use of MsOH in dioxane seems to

11) K. Suzuki and N. Endo, *Chem. Pharm. Bull.* (Tokyo), **25**, 1143 (1977).
12) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1857 (1974).
13) M.A. Ondetti, J. Pluscec, E.R. Weaver, N. William, E.F. Sobo and O. Kocy, "Chemistry and Biology of Peptides," ed. by J. Meienhofer, Ann Arbor Sci. Publ. Inc., Ann Arbor, 1972, p. 525.
14) W. König and K. Geiger, *Chem. Ber.*, **103**, 788 (1970). This reagent was treated with activated charcoal in water and recrystallized before use. Because the use of the reagent stored long term gave a coloured by-product in the desired peptide derivatives.

be preferable because the cleavage velocity of the Boc group is a little faster than that of TsOH in dioxane as shown in Table II.

Experimental

All melting points are uncorrected. Ether and dioxane used were free from water and peroxide. Unless otherwise mentioned, Boc group of the protected peptide was deblocked with 2 M TsOH in dioxane, and paper chromatography was performed on Toyo Roshi No. 51 with the following solvent systems: *Rf*(A), BuOH–AcOH–H_2O (4:1:5, upper layer);[15] *Rf*(B), BuOH–AcOH–pyridine–H_2O (15:3:10:12).[16] Amino acid analysis was carried out on a Hitachi Model KLA-3B amino acid analyzer according to the directions given by Moore, *et al.*[17]

Determination of Tryptophan——Treatment of Boc-Trp-OH (0.01 mmol) with 2 M TsOH in dioxane (10 eq) containing 2% anisole was performed at room temperature. At the four intervals, 5, 15, 30 min and 24 hr, the reaction mixture was neutralized with 1 N $NaHCO_3$ and diluted with pH 2.2 citrate buffer under cooling. A part of the solution (1.0 μmol) was applied to amino acid analyzer. Tryptophan was similarly determined with the use of the other reagents shown in Table I. The results are shown in Table I. When 2.5 M MsOH in dioxane, containing 2% anisole and 2% TPP were mixed 24 hr before use, the recovery of tryptophan from the reaction mixture of Boc-Trp-OH with the reagent was 81%.

Treatment of Boc-Trp-OH with 2 M TsOH in Dioxane Containing Anisole or 3 M MsOH in AcOH Containing Anisole and EDT——Boc-Trp-OH (300 mg) was dissolved in 2 M TsOH in dioxane (4 ml) containing 2% anisole and allowed to stand at room temperature for 30 min. The reaction mixture was diluted with ether and the precipitate thereby formed was collected by centrifugation and washed with ether. The resulting product in H_2O (5 ml) was washed with EtOAc and added to a column (1.8×15 cm) of Dowex 1×2 (acetate form), which was eluted with H_2O. The fractions positive to ninhydrin and Ehrlich reagents were pooled, evaporated to dryness and lyophilized. After neutralization with 1 N NH_4OH, lyophilization was repeated to constant weight; yield 195 mg (97%).

Treatment with 3 M MsOH in AcOH (3 ml) containing 2% anisole and 2% EDT was carried out in the same manner described above; yield 197 mg (98%). UV spectrum in 0.1 N HCl or 0.1 N NaOH was measured with a Hitachi Model 124, IR spectrum in nujol was measured with a Hitachi Model EPI-G_2 and MS was measured with a Hitachi Model RMU-6MG.

Properties of the Reagents to Various Protecting Groups——Treatment of amino acid derivatives (0.1 mmol each), Boc-Lys(Z)-OH, Boc-Asp(OBzl)-OH and H_2^+-Gly-OBzl·TsO^-, with the reagents (10 eq) shown in Table I was performed at room temperature. At two intervals, 30 min and 24 hr, a part of the reaction mixture was examined by paper chromatography. Products were stained by ninhydrin reagent.

Effect of the Reagents in Dioxane to Ester Bond of Amino Acid with Merrifield's Resin——Boc-Lys(Z)-resin (100 mg, 0.245 mmol of Lys/g), which was prepared according to the procedure by Suzuki, *et al.*[11] was treated with 2 M TsOH in dioxane (5 ml) containing 2% anisole at room temperature for 20 hr. The resin was then washed with the following solvents (10 ml each) respectively; dioxane, DMF, EtOH, H_2O and EtOH, on filter and dried to constant weight in vaccum. The resin thus obtained was hydrolized with the mixture of conc. HCl-propionic acid, according to the procedure by Robinson, *et al.*[18] The lysine content in the resin was 0.242 mmol/g of resin, as determined on amino acid analyzer. Similarly, the lysine contents in the resin treated with 2.5 M MsOH in dioxane, 3 M EsOH in dioxane and 50% TFA in CH_2Cl_2 were 0.240, 0.238 and 0.220 mmol/g of resin respectively.

Identification of By-products in the Reaction Mixture of Tryptophan and Methionine Derivatives in the Presence of Z(OMe) Group——Amino acid derivatives (0.1 mmol each), Boc-Trp-OH, Z(OMe)-Trp-OH, Z(OMe)-Gly-OH, Boc-Met-OH and Z(OMe)-Met-OH, or their mixture, were treated with 2 M TsOH in dioxane (10 eq) containing 2% anisole at room temperature for 30 min. A part of the reaction mixture was examined by paper chromatography. Products were stained by ninhydrin or Ehrlich reagent and the results were shown in Fig. 1.

Swelling Text of Sulfonic Acid in Dioxane Reagents to Boc-amino Acyl-resin——Sulfonic acid in dioxane reagents shown in Table I and 50% TFA in CH_2Cl_2 (5 ml each) were added to Boc-Val-resin (500 mg, 0.339 mmol of Val/g). After 2 hr, the resin was collected on cylindrical tube filter and the volume of the resin was measured. The volume was 2.0 ml in 2 M TsOH, 2.0 ml in 2.5 M MsOH, 1.9 ml in 3 M EsOH in dioxane and 1.9 ml in 50% TFA in CH_2Cl_2 respectively.

Cleavage of the Boc Group in Boc-Gly- and Boc-Val-resin with TsOH and MsOH in Dioxane——Boc-Gly-resin (0.122 mmol of Gly/g) was prepared according to the procedure by Suzuki, *et al.*[11] 2 M TsOH and 2.5 M MsOH in dioxane containing 2% anisole were used as de-Boc reagent and deprotection reaction

15) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
16) S. G.Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).
17) S. Moore, D.H. Spackman and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).
18) J. Scotchler, R. Lozier and A.B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970).

times were 15, 30 and 60 min each. The liberated amino group was determined by the procedure given by Hancock, *et al.*[10] Boc-Val-resin (0.339 mmol of Val/g)[19] was similarly treated. The results were shown in Table II.

Boc-Ser(Bzl)-Trp-Gly-OBzl (I)——Boc-Trp-Gly-OBzl[11] (450 mg) was dissolved in AcOH (0.5 ml) and 3 M MsOH in AcOH (2 ml) containing 2% anisole and 2% EDT. After standing at room temperature for 30 min, the reaction mixture was diluted with ether. The oily product thereby formed was collected by centrifugation, washed with ether and reprecipitated from MeOH and ether. The title compound was prepared from the product thus obtained and Boc-Ser(Bzl)-OH (295 mg) with the use of DCC and HONB as coupling reagent and the reaction mixture was treated in a similar manner described in a previous paper.[20] The resulting product was reprecipitated from EtOAc and petroleum ether; yield 628 mg (100%); mp 65—71°; $[\alpha]_D^{13}$ −7.8° (c=0.9, DMF); *Rf*(A) 0.85, *Rf* (B) 0.95, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{35}H_{40}N_4O_7$: C, 66.86; H, 6.41; N, 8.91. Found; C, 66.81; H, 6.35; N, 8.77.

Boc-Phe-Ser(Bzl)-Trp-Gly-OBzl (II)——I (368 mg) was treated with 2 M TsOH in dioxane (2 ml) containing 2% anisole at room temperature for 30 min. The reaction mixture was worked up in the same manner described above. The title compound was prepared from the product thus obtained and Boc-Phe-OH (155 mg) as described for the preparation of I; yield 279 mg (61% based on I used); mp 95—106°; $[\alpha]_D^{13}$ +12.0° (c= 0.5, DMF); *Rf*(A) 0.81, *Rf*(B) 0.90 single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{44}H_{49}N_5O_8$: C, 68.11; H, 6.37; N, 9.03. Found: C, 67.88; H, 6.62; N, 8.82.

Z-Arg(MBS)-Phe-Ser(Bzl)-Trp-Gly-OBzl (III)——This compound was prepared from II (223 mg) and Z-Arg(MBS)-OH[21] (138 mg) as described for the preparation of II; yield 235 mg (72%); mp 105—115°; $[\alpha]_D^{12}$ 0° (c=0.8, DMF). *Anal.* Calcd. for $C_{60}H_{65}N_9O_{12}S$: C, 63.42; H, 5.77; N, 11.10. Found: C, 63.33; H, 5.96; N, 11.38.

Boc-Arg(NO_2)-Trp-Gly-OEt(IV)——This compound was prepared from Boc-Trp-Gly-OEt[22] (778 mg) and Boc-Arg(NO_2)-OH (638 mg) as described for the preparation of II. Coupling reagent was DCC and HOBt. The combined product which was appeared as solid in part during washing of EtOAc extracts and obtained from the EtOAc solution was reprecipitated from CH_3CN and ether: yield 1.0 g (85%); mp 121°; $[\alpha]_D^{15}$ − 16.0° (c=1.0, DMF); *Rf*(A) 0.57, *Rf*(B) 0.89, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{26}H_{38}N_8O_8$: C, 52.87; H, 6.48; N, 18.97. Found: C, 53.05; H, 6.75; N, 18,74.

Boc-Phe-Arg(NO_2)-Trp-Gly-OEt (V)——This compound was prepared from IV (295 mg) and Boc-Phe-OH (133 mg) as described for the preparation of IV. The product was reprecipitated from CH_3CN and ether; yield 278 mg (75%); mp 120—125°; $[\alpha]_D^{15}$ −23.0° (c=1.0, DMF); *Rf*(A) 0.69. *Rf*(B) 0.93, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{35}H_{47}N_9O_9$: C, 56.98; H, 6.42; N, 17.09. Found: C, 57.09; H, 6.67; N, 16.99.

Boc-Phe-Arg(NO_2)-Trp-Gly-OH[23] (VI)——To a solution of V (90 mg) in MeOH (1 ml), 1 N NaOH (0.124 ml) was added and stirred at room temperature for 1 hr. The reaction mixture was worked up in the usual manner. The product was reprecipitated from EtOAc and petroleum ether; yield 75 mg (87%); mp 155—156° (dec.); $[\alpha]_D^{15}$ −20.6° (c=0.9, MeOH) (lit.[23] mp 155—156° (dec.); $[\alpha]_D^{26}$ −22.3° (c=1.0, MeOH)); *Rf*(A) 0.65, *Rf*(B) 0.72, single spot positive to ninhydrin and Ehrlich reagents.

H_2^+-Ala-Pro-OBzl·TsO$^-$ (VII)——Boc-Ala-Pro-OBzl[24] (370 mg) was dissolved in 2 M TsOH in dioxane (3 ml) and stirred at room temperature for 30 min. Ether was added to the reaction mixture to give crystals. The product was collected, washed with ether and reprecipitated from MeOH and ether; yield 530 mg (97%); mp 156—157°; $[\alpha]_D^{15}$−80.0° (c=1.0, DMF); *Rf*(A) 0.89, *Rf*(B) 0.90, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{22}H_{28}N_2O_6S$: C, 58.91; H, 6.29; N, 6.24. Found: C, 58.76; H, 6.38; N, 5.98.

Boc-Trp-Ala-Pro-OBzl (VIII)——This compound was prepared from VII (1.49 g) and Boc-Trp-OH (0.83 g) as described for the preparation of IV; yield 1.52 g (100%); mp 85—105°; $[\alpha]_D^{15}$ −60.0° (c=1.0, DMF); *Rf*(A) 0.77, *Rf*(B) 0.86, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{31}H_{38}N_4O_6$: C, 66.17; H, 6.81; N, 9.96. Found; C, 66.40; H, 7.19; N, 9.71.

Boc-Lys(Z)-Trp-Ala-Pro-OBzl (IX)——This compound was prepared from VIII (560 mg) and Boc-Lys(Z)-OH (380 mg) as described for the preparation of IV; yield 707 mg (86%); mp 84—103°; $[\alpha]_D^{15}$ −44.0° (c=1.0, DMF); *Rf*(A) 0.66, *Rf*(B) 0.74, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{45}H_{56}N_6O_9$: C, 67.99; H, 7.10; N, 10.57. Found: C, 67.87; H, 6.90; N, 10.33.

Boc-Gln-Lys(Z)-Trp-Ala-Pro-OBzl (X)——This compound was prepared from IX (275 mg) and Boc-Gln-ONp (110 mg) in the usual manner. The resulting product was reprecipitated from EtOAc and petroleum ether; yield 237 mg (75%); mp 109—111°; $[\alpha]_D^{15}$ −53.0° (c=1.1, DMF); *Rf*(A) 0.83, *Rf*(B) 0.88, single spot

19) R.B. Merrifield, *J. Am. Chem. Soc.*, **86**., 304 (1964).
20) K. Suzuki, K. Nitta and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 3025 (1976).
21) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **24**, 1568 (1976).
22) M. Chorev and Y.S. Klausner, *J. Chem. Soc., Chem. Comm.*, **1976**, 596.
23) M. Fujino, O. Nishimura and C. Hatanaka, *Chem. Pharm. Bull.* (Tokyo), **17**, 2135 (1969).
24) G.P. Lorenzi, B.B Doyle and E.R. Blout, *Biochemistry*, **10**, 3046 (1971).

positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{50}H_{64}N_8O_{11}\cdot H_2O$: C, 61.84; H, 6.85; N, 11.54. Found: C, 62.08; H, 6.98; N, 11.50.

Acknowledgement The authors are grateful to Professor Y. Yamamoto of this college for helpful advice. Thanks are also due to the staff of Central Analysis Laboratory, Tohoku University, for elemental analysis.